# PATENT COOPERATION TREATY

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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# PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 23.09.2004 |
| Applicant's or agent's file reference | **IMPORTANT NOTIFICATION** |
| PA0248-PCT | |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/GB 03/02983 | 10.07.2003 | 18.07.2002 |

Applicant
AMERSHAM BIOSCIENCES UK LIMITED

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

HSL ✓
IB

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# PATENT COOPERATION TREATY

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference PA0248-PCT | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No. PCT/GB 03/02983 | International filing date *(day/month/year)* 10.07.2003 | Priority date *(day/month/year)* 18.07.2002 |
| International Patent Classification (IPC) or both national classification and IPC C12Q1/68 | | |
| Applicant AMERSHAM BIOSCIENCES UK LIMITED | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 23.01.2004 | 23.09.2004 |
| Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016 | Authorized Officer Gunster, M Telephone No. +31 70 340-4412 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/02983

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

| | |
|---|---|
| 1-5, 9-12, 14-27 | as originally filed |
| 6-8, 13 | received on 30.06.2004 with letter of 30.06.2004 |

**Claims, Numbers**

| | |
|---|---|
| 1-20 | received on 30.06.2004 with letter of 30.06.2004 |

**Drawings, Sheets**

| | |
|---|---|
| 1/13-13/13 | as originally filed |

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 21-23

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-19 |
| | No: | Claims | 20 |
| Inventive step (IS) | Yes: | Claims | 1-19 |
| | No: | Claims | 20 |
| Industrial applicability (IA) | Yes: | Claims | 1-20 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

**Re Item V**

Reference is made to the following documents:
D1: WO 00 68661;
D2: WO 98 45704.

NOVELTY

The subject-matter of claims 1-19 is new in the sense of Article 33(2) PCT because the prior art does not disclose a method for determining the function of effect of a genetic element or a chemical modulator from a library of said genetic elements and chemical modulators of known and unknown function on a population of cells comprising determining the distribution of an indicator nucleic acid in the presence of an effector nucleic acid sequence and a first and second chemical modulator.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 20 is not new in the sense of Article 33(2) PCT.
Document D2 (claim 74) discloses an automated system together with an imaging system and a computerized data processing device, which is *suitable for* determining the function or effect of a chemical and/or genetic element on a population of cells by performing the method according to claims 1-19.

INVENTIVE STEP

The present application meets the criteria of Article 33(3) PCT, because the subject-matter of claims 1-19 involves an inventive step.

The document D1 (page 27 line 23 - page 31, line 20) is regarded as being the closest prior art to the subject-matter of claims 1-19 and discloses a method for determining the function of effect of a chemical modulator from a library of chemical modulators of known and unknown function on a population of cells comprising determining the distribution of an indicator nucleic acid in the presence of a first and second chemical modulator.
The subject-matter of claims 1-19 differs from this known method in that a library of effector nucleic acids is co-expressed.
The problem to be solved by the present invention may therefore be regarded as the provision of an method in which simultaneously genetic elements and chemical

modulators can be tested for their effect on the distribution of an indicator nucleic acid.

The solution proposed in claims 1-19 of the present application is considered as involving an inventive step (Article 33(3) PCT) for the following reasons. There is no hint in the prior art that suggests the provision of a method for simultaneously determining the effect of genetic elements and chemical modulators on the distribution of an indicator nucleic acid. Consequently, the subject-matter of claims 1-19 is not obvious to the skilled person and therefore involves an inventive step.

## INDUSTRIAL APPLICABILITY

The subject-matter of claims 1-20 is industrially applicable in the field of molecular biology.

each collection which are of known function, it is possible to assign function to previously uncharacterised elements by linkage to known elements.

Thus the method of the present invention allows function to be assigned at a molecular and temporal level for any cellular component, chemical, drug or other active moiety which induces a change in behaviour of an endogenous or exogenous cellular component by reference to changes induced by other moieties of known function. Non-destructive single cell analytical methods are used to analyse the cellular behaviour of indicators influenced by genetic effectors and chemical modulators, where the indicators and effectors may be either endogenous or exogenous to the cell.

**Summary of the Invention**

According to a first aspect of the present invention, there is provided a method for determining the function or effect of a genetic element or a chemical modulator from a library of genetic elements and chemical modulators of known and unknown function on a population of cells, the method comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in the cells in the presence and the absence of a first chemical modulator, which modulator affects the distribution of the indicator, wherein the cells are both co-expressing an effector nucleic acid sequence and are in the presence of a second chemical modulator; and

ii) analysing the distribution data from all combinations of the effector, modulator and indicator to derive functional linkages and assign function to the effector and the second modulator.

In the context of the present invention, the following terms are to be interpreted as defined below:

'*Effector*' – a nucleic acid sequence with biological function or activity, resulting either from an expressed protein with biological function or activity (e.g. cDNA or other coding nucleic acid sequence) or resulting from another mechanism of action (e.g. antisense and RNAi sequences);

'*Modulator*' - a chemical moiety with biological function or activity;

'*Indicator*' - a nucleic acid sequence which comprises a detectable label, encodes a detectable label or which may optionally be fused to a sequence encoding a detectable protein label and expressed in a cell resulting in a characteristic localisation of the detectable protein;

'*Cellular Assay*' - an assay providing a diagnostic read-out of the biological activity of an effector or modulator

In a second aspect of the present invention, there is provided a method for determining the function or effect of a genetic element or a chemical modulator from a library of said genetic elements and chemical modulators of known and unknown function on a population of cells, the method comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in said cells in the presence of a first chemical modulator, which modulator affects the distribution of the indicator, wherein the cells are both co-expressing an effector nucleic acid sequence and are in the presence of a second chemical modulator;

ii) comparing the distribution data of i) above with known distribution data, stored on an electronic or optical database, for the indicator nucleic acid sequence in the absence of the first chemical modulator; and

iii) analysing the distribution data from all combinations of the effector, modulator and indicator to derive functional linkages and assign function to the effector and the second modulator.

Suitably, the effector nucleic acid sequence encodes a protein or peptide and is selected from the group consisting of DNA, cDNA, RNA and Protein Nucleic Acid.

Preferably, the effector nucleic acid sequence is an antisense oligonucleotide (cf. Dean (2001) Current Opinion in Biotechnology, *12*, 622-625). More preferably, the effector nucleic acid is a small interfering RNA (siRNA) which causes gene silencing (cf. Elbashir *et al.* (2002) Methods, *26*, 199-213). RNA interference (RNAi) is a highly conserved gene silencing mechanism that uses double-stranded RNA as a signal to trigger the degradation of homologous mRNA. The mediators of sequence-specific mRNA degradation are 21- to 23-nt small siRNAs generated by ribonuclease III cleavage from longer double-stranded RNA.

Preferably, there is provided an expression vector comprising suitable expression control sequences operably linked to an indicator or an effector nucleic acid sequence according to the present invention. The DNA construct of the invention may be inserted into a recombinant vector, which may be any vector that may conveniently be subjected to recombinant DNA procedures. The choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, ie. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

Preferably, the localisation of the detectable label is determined using an imaging system. A suitable Imaging System is the In Cell Analyzer, as described in WO 99/47963 and PCT/GB03/01816.

In a third aspect of the present invention, there is provided an automated system for determining the function or effect of a chemical and /or a genetic element on a population of cells comprising use of the method as hereinbefore described together with an imaging system and a computerised data processing device.

## Brief Description of the Invention

Figure 1; Schematic for generation of an indicator cell assay from a cDNA collection.

Figure 2; Schematic for establishing an inferred functional relationship between an effector and a modulator in a cellular assay.

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## Claims

1. A method for determining the function or effect of a genetic element or a chemical modulator from a library of said genetic elements and chemical modulators of known and unknown function on a population of cells comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in said cells in the presence and the absence of a first chemical modulator, which modulator affects said distribution of said indicator, wherein the cells are both co-expressing an effector nucleic acid sequence and are in the presence of a second chemical modulator; and

ii) analysing the distribution data from all combinations of said effector, modulator and indicator to derive functional linkages and assign function to the effector and said second modulator.

2. A method for determining the function or effect of a genetic element or a chemical modulator from a library of said genetic elements and chemical modulators of known and unknown function on a population of cells comprising

i) determining the distribution of an indicator nucleic acid sequence being expressed in said cells in the presence of a first chemical modulator, which modulator affects said distribution of said indicator, wherein the cells are both co-expressing an effector nucleic acid sequence and are in the presence of a second chemical modulator;

ii) comparing the distribution data of i) above with known distribution data, stored on an electronic or optical database, for the indicator nucleic acid sequence in the absence of said first chemical modulator; and

iii) analysing the distribution data from all combinations of said effector, modulator and indicator to derive functional linkages and assign function to the effector and said second modulator.

3. The method according to either of claims 1 or 2, wherein the effector nucleic acid sequence encodes a protein or peptide and is selected from the group consisting of DNA, cDNA, RNA and Protein Nucleic Acid.

4. The method according to any of claims 1 to 3, wherein the effector nucleic acid is an antisense oligonucleotide.

5. The method according to any of claims 1 to 3, wherein the effector nucleic acid is a small interfering RNA (siRNA) which causes gene silencing.

6. The method according to any of claims 1 or 5, wherein the effector nucleic acid comprises a nucleic acid sequence in a cellular expression vector.

7. The method of claim 6, wherein said expression vector is selected from the group consisting of plasmid, retrovirus and adenovirus.

8. The method according to any of claims 1 to 7, wherein the indicator nucleic acid sequence comprises a detectable label or encodes a detectable label.

9. The method according to claim 8, wherein the indicator nucleic acid sequence is created by fusing the effector sequence to a nucleic acid sequence encoding a detectable label.

10. The method according to either of claims 8 or 9, wherein said detectable label is selected from the group consisting of fluorescent protein, enzyme, antigen and antibody.

11. The method according to claim 10, wherein said fluorescent protein is a modified Green Fluorescent Protein (GFP) having one or more mutations selected from the group consisting of Y66H, Y66W, Y66F, S65T, S65A, V68L, Q69K, Q69M, S72A, T203I, E222G, V163A, I167T, S175G, F99S, M153T, V163A, F64L, Y145F, N149K, T203Y, T203Y, T203H, S202F and L236R.

12. The method according to claim 11, wherein said modified GFP has three mutations selected from the group consisting of F64L-V163A-E222G, F64L-S175G-E222G, F64L-S65T-S175G and F64L-S65T-V163.

13. The method according to claim 10, wherein said enzyme is selected from the group consisting of β-galactosidase, nitroreductase, alkaline phosphatase and β-lactamase.

14. The method according to any of claims 1 to 13, wherein the modulator is selected from the group consisting of organic compound, inorganic compound, peptide, polypeptide, protein, carbohydrate, lipid, nucleic acid, polynucleotide and protein nucleic acid.

15. The method according to any of claims 1 to 14, wherein the modulator is selected from a combinatorial library comprising similar organic compounds such as analogues or derivatives.

16. The method according to any of claims 1 to 15, wherein said cell is an eukaryotic cell.

17. The method according to claim 16, wherein said eukaryotic cell is selected from the group consisting of mammal, plant, bird, fungus, fish and nematode, which cell may or may not be genetically modified.

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18. The method according to claim 17, wherein said mammalian cell is a human cell, which cell may or may not be genetically modified.

19. The method according to any of claims 1 to18, wherein the distribution of the indicator nucleic acid is determined using an imaging system.

20. An automated system for determining the function or effect of a chemical and /or a genetic element on a population of cells comprising use of the method according to any of claims 1 to 19 together with an imaging system and a computerised data processing device.